REMARKS

After entry of the amendments made herein , claims 1 – 6, 8 –15, 17- 22, 25 – 29 and 31 – 33 and 35-38 are pending in the application. Claims 1-3, 8, 9, 11, 15, and 17 – 22 are herein amended. Claims 7, 16, 23, 24, 29, 30, and 34 have been cancelled. New claims 36-38 are herein added. No new matter has been added by virtue of the amendments or new claims, support being found throughout the specification and from the claims as filed. Specifically, support for the amendment to claim 1 can be found on page 14 of the specification.

Applicant's representatives thank Examiner McGillem and Examiner Woitach for their time and helpful discussion in the telephonic interview of November 2, 2007. Examiner McGillem, Examiner Woitach and Applicant's representatives, Alexandra Jones and Jeffrey Kopacz, participated in the telephone interview. The interview concerned the substance of the outstanding Office Action, and is summarized herein.

Objections

Applicants thank the Examiner for reconsideration and withdrawal of the objection to the oath.

Rejections

35 U.S.C. § 112, second paragraph

Claim 29 has been rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for allegedly failing to point out and distinctly claim the subject matter which applicant regards as the invention. Applicants respectfully traverse the rejection.

The Examiner argues that "claim 29 is vague and indefinite because it is drawn to the treatment of a tumor using the method of claim 1, but claim 1 is an *in vitro* method. As the claim is written, it does not comprise limitations regarding how *in vitro* electrofused cell will be used to treat a tumor." (Office Action, p.2 – 3).

Instant claim 29 has been cancelled. Applicants respectfully request withdrawal of the rejection.

35 U.S.C. § 112, first paragraph written description

Claim 29 has been rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement. The Examiner argues that "claim 29 is a method for treatment of a tumor comprising using the method according to claim 1, but claim 1 has been amended to limit the method to an *in vitro* method. Therefore, in order to use the method of claim 29 comprising using an in vitro method, the skilled artisan would have to practice the method of electrofusion of two fusion partners having cell-like membranes *in vitro* and then use the electrofused partners to treat a tumor which encompasses an *ex vivo* treatment method." (Office Action, p.3). The Examiner argues that this "constitutes impermissible new matter." (Office Action, p.4).

Applicants disagree with the Examiner, but in order to expedite prosecution have herein canceled claim 29. Applicants respectfully request withdrawal of the rejection.

35 U.S.C. § 112, first paragraph enablement

The Examiner has rejected claims 1 – 6, 8 – 22, 25 – 28, 31 – 33 and 35 under 35 U.S.C. § 112, first paragraph, because the specification, "while being enabling for *in vitro* selective electrofusion of at least two fusion partners having cell-like membranes, does not reasonably provide enablement for conducting in vitro fertilization by selective electrofusion of an egg cell or an enucleated egg cell and a sperm cell at any development stage, or for conducting non-human cloning." (Office Action, p.4). Applicants respectfully traverse the rejection.

The instant claims recite an *in vitro* method for selective electrofusion of a mammalian cell and a fusion partner having a cell-like membrane, comprising A) selecting the mammalian cell and the fusion partner; bringing into contact the mammalian cell and the fusion partner[s]; providing an electric field using at least one microelectrode, which is of a strength sufficient to obtain fusion of the mammalian cell and the fusion partner, and highly focused on the mammalian cell and the fusion partner[s], wherein said at least one microelectrode is positioned by use of a microscope, at least one micropositioner and/or a stereotactic device, wherein at least one microelectrode is sufficiently small to permit the selective fusion of the mammalian cell

and the fusion partner, and the highly focused electric field minimizes the risk for unwanted fusion of surrounding cells (claim 1). In other embodiments, the other fusion partner is selected from the group consisting of a single cell, a liposome, a proteoliposome, a synthetic vesicle, an egg cell and an enucleated egg cell. Sperm cells have been deleted from those claims dependent on claim 1. New claim 38 is herein added. New claim 38 describes a method similar to claim 1 but requires a target cell and a fusion partner, wherein the target cell and fusion partner are selected from the group consisting of a single mammalian cell, a liposome, a proteoliposome and a synthetic vesicle.

The Examiner indicates that the invention is enabled for *in vitro* selective electrofusion of at least two fusion partners having cell-like membranes. This would include egg cells which have cell-like membranes.

As pointed out in the previous Office Action, according to the invention as instantly claimed, it would not require a great deal of work to perform an *in vitro* method for selective electrofusion of at least two fusion partners (mammalian cell and fusion partner having a cell-like membrane). The specification provides teaching of electrical field strength and number (p.8), strength and duration of fusion pulse (p.8), positioning of the electrodes (p.9), and preferred dimension of the electrodes (p.9). The specification teaches preparation of fluorescence encapsulated vesicles for use in the method (p.16), and chemicals and materials needed to perform the method (p.17 – 18). The specification provides examples of experimental setup and instrumentation (p.14 – 15). The specification provides working examples of both cell-cell and cell-liposome fusion (p.18 – 21). The instant specification provides ample guidance to perform an *in vitro* method for selective electrofusion of a mammalian cell and a fusion partner having a cell-like membrane. Any experimentation required to perform the method using, for example, any mammalian cell and a fusion partner having cell-like membrane in an *in vitro* environment would not require more than routine experimentation.

In *In re Wands*, the court stated that "[e]nablement is not precluded by the necessity for some experimentation, such as routine screening. However, experimentation needed to practice the invention must not be undue experimentation. 'The key word is 'undue' not 'experimentation' (citing *In re Angstadt*, 537 F. 2d 498 at

504, 190 U.S.P.Q. 214 at 219 (C.C.P.A. 1976)). The Court also stated that "the test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." (citing *In re Jackson*, 217 U.S.P.Q. 804 at 807 (Bd. App. 1982)), emphasis added. Thus, the instant specification provides ample guidance to perform an *in vitro* method for selective electrofusion of a mammalian cell and a fusion partner having a cell-like membrane. Any experimentation required to perform the method using, for example, a mammalian cell and a fusion partner having a cell-like membrane in an *in vitro* environment would not require more than routine experimentation.

The common physiology between mammalian cell membranes allows the use of the claimed selective electrofusion method with diverse cell types. In addition, bulk electrofusion is a well studied technique that has been used with numerous cell types, including egg cells (e.g., See background of the invention). As such the present method of selective electrofusion can be practiced without undue experimentation for any mammalian cell, including egg cells.

Even if some species of mammalian cells can not be electrofused with the claimed method, this would not constitute undue experimentation. The Federal Circuit has held that claims may encompass some inoperative species, so long as the number of inoperative species does not become significant and force one of ordinary skill into undue experimentation in order to practice the invention (*Atlas Powder Co. v. E.I. du Pont de Nemours & Co.*, 750 F.2d 1569, 224 U.S.P.Q. 409 (Fed. Cir. 1984)). That is not the case here. The numerous cell populations for which bulk electroporation has been shown to be effective, in combination with the numerous selective electrofusion working examples contained herein fully enable the use of the claimed method with a mammalian cell and fusion partner having a cell-like membrane.

Accordingly, in view of the amended claims, applicants respectfully request withdrawal of the rejection and allowance of the claims.

The Examiner has rejected claim 29 under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the enablement requirement. The Examiner argues that

the claim contains subject matter that was not described in the specification in such a way as to enable one of skill in the art…to make and/or use the invention." (Office Action, p.10). Applicants respectfully traverse the rejection. However, in order to expedite prosecution Applicants have herein canceled claim 29. Applicants respectfully request withdrawal of the rejection.

Claim Rejections- 35 U.S.C. § 102(b)

Claims 1 and 17 have been rejected under 35 U.S.C. § 102(b) as being anticipated by Magae et al. (Appl. Micro. Biotechol., 1986, Vol 24, 509-511). The Examiner argues that the Magae et al. reference teaches a method to fuse two giant plant protoplasts by using two glass electrodes prepared from glass capillaries and attached to a micromanipulator. Applicants respectfully traverse the rejection.

Applicants have herein amended claim 1 to more clearly define the invention. As amended, instant claim 1 recites an *in vitro* method for selective electrofusion of a mammalian cell and a fusion partner having a cell-like membrane, comprising selecting the mammalian cell and the fusion partner; bringing into contact the mammalian cell and the fusion partner[s]; providing an electric field using at least one microelectrode, which is of a strength sufficient to obtain fusion of the mammalian cell and the fusion partner, and highly focused on the mammalian cell and the fusion partner[s], wherein said at least one microelectrode is positioned by use of a microscope, at least one micropositioner and/or a stereotactic device, wherein at least one microelectrode is sufficiently small to permit the selective fusion of the mammalian cell and the fusion partner, and the highly focused electric field minimizes the risk for unwanted fusion of surrounding cells. Claim 17 depends from claim 1, and recites that the target cell or the fusion partners are provided in a buffer prior to step B.

In order to anticipate a claim, each and every element of the claim must be found in a single reference. This is discussed in the Manual of Patent Examining Procedure § 2131. The Magae reference neither teaches nor suggests an *in vitro* method for **selective electrofusion of a mammalian cell and a fusion partner** having a cell-like membrane, comprising **selecting the mammalian cell and the fusion partner**; **bringing into contact the mammalian cell and the fusion partner**[s] and providing an electric field

using at least one microelectrode, which is of a strength sufficient to obtain fusion and **highly focused on the target cell** and fusion partner.

The instant invention is based on a method that allows selective and controllable fusion of a **mammalian cell** and a fusion partner. The Magae reference teaches fusion of two giant plant protoplasts using glass electrodes attached to a micromanipulator, and made more efficient by manipulation of the conditions and size of the cell. In order for protoplast electrofusion, the protoplasts had to be enlarged by pre-treatment protocol [Mage et al., p. 510, first column]. Furthermore, Mage et al. states that "[t]his method can, however, only be used for protoplasts large enough for the introduction of microelectrodes." [Mage et al. p. 511, first column]

Accordingly, Applicants respectfully request withdrawal of the rejection and allowance of the claims.

Claim Rejections- 35 U.S.C. § 102(e)

Claims 1 – 2, 8 – 12, 15 – 19, and 26 – 29 have been rejected under 35 U.S.C. § 102(e) as being anticipated by Pui et al., US Patent No. 6,093,557 ("the '557 reference"). Applicants respectfully submit that the invention as claimed is not anticipated by the '557 reference and respectfully traverse the rejection.

Instant claim 1 has been described above. Claims 2, 8 – 12, 15 – 19 and 26 and 27 depend from claim 1. Claim 29 has been cancelled.

The teachings of the '557 reference do not anticipate the claimed method. Specifically, the '557 reference does not teach the fusion of a single pair of fusion partners at a given time. It does not teach the **selective electrofusion** of a mammalian cell and a fusion partner having a cell-like membrane, comprising **selecting the mammalian cell and the fusion partner**; **bringing into contact the mammalian cell and the fusion partner**[s] and providing an electric field using at least one microelectrode, which is of a strength sufficient to obtain fusion and **highly focused on the target cell** and fusion partner wherein at least one microelectrode is sufficiently small to permit the selective fusion of the mammalian cell and the fusion partner, and **the highly focused electric field minimizes the risk for unwanted fusion of surrounding cells**.

The Examiner argues that "it appears that the issues surround these rejections are based in part on the limitation of 'highly focused' (and) (t)he limitation of highly focused has been discussed in the above rejection." (Office Action, p.21). The Examiner argues that "the specification does not provide a limiting definition of the phrase 'selective electrofusion' and the Applicants have not pointed to disclosure that supports a limiting definition of the phrase 'selective electrofusion.'" (Office Action, p.21).

As detailed above, Applicants submit that in the instant claims, the phrase a method for selective electrofusion of a mammalian cell and a fusion partner having a cell-like membrane is fully supported by the disclosure. Applicants submit that the meaning of the term "highly focused" is well-supported and described in the specification. For example, on page 8, lines 30 – 37, the specification teaches:

> The electrical field used in step B to obtain fusion should be *highly focused in order to avoid affecting any surrounding structures*...To focus the electrical field it is preferable to provide the electrical field by use of one or two microelectrodes positioned close to the two fusion partners, i.e. 0 – 10 μm, preferably 0 – 5 μm, from the cellular membrane. (emphasis added)

The term "highly focused" is made even clearer when read in light of the preceding paragraphs on page 8 that describe how the electrical field is obtained (lines 8 - 30), as well as the Examples. In particular, Example 3 (p.22) teaches positioning for cell fusion using single open-bore capillaries, and specifically positioning of the capillary tip using micromanipulators and fusion of two aligned cells by applying pulses of 5 to 15 kV for 0.1 – 5 seconds. Furthermore, page 14, lines 23-28 state:

> An advantage of this set-up is that the electrodes are of cellular to subcellular dimensions, enabling fusion of single cells in complex cellular networks grown on a substratum. **At the same time, the highly focused electric field minimizes the risk for unwanted fusion or electroporation of surrounding cells.** [Emphasis Added]

Applicants have pointed to specific passages and Examples in the disclosure where this language finds support. Moreover, Applicants have amended the claims to indicate that the highly focused electric field minimizes the risk for unwanted fusion of surrounding cells.

The '557 reference fails to teach a method for selective electrofusion of a mammalian cell and at least one fusion partner having a cell-like membrane. The Examiner argues that the '557 reference teaches "that the spray can be confined to one or more target cells." (Office Action, p.21). This is not what is taught by the '557 reference. The '557 does not teach the selectively electrofusion of a single pair of fusion partner. The method of fusion taught by the '557 "uses an electrospraying apparatus to establish a spray of charged particles." (col 4, line 51). The '557 reference teaches that "the concentration of charged particles in the spray is in the range of about 10^5 particles/ cubic centimeter (particles/cc) to about 10^{12} particles/ cc…blow about 10^5 particles/ cc, the concentration of particles is too low for the space charge effect to attain a velocity for introduction into most target cells. (col 5, line 63 – col 6 line 2). Thus, the spray of particles taught by the '557 reference does not provide selectivity of fusion between a target cell and a fusion partner, but rather a method involving "a spray of substantially dispersed particles (claim 1)" wherein one or more of the substantially dispersed particles is introduced into the target cell.

Further, the '557 reference does not provide an electric field that is highly focused on the mammalian cell and the fusion partner to cause the electrofusion of a mammalian cell and fusion partner. In fact, the '557 does not even teach "electrofusion" as known by one of skill in the art. Rather the '557 teaches the release of particles having sufficient velocity to forcibly penetrate target cells. An electrode is used only "to provide a nonuniform electric field for establishing a charged spray and also provides direction for the particles of the charged spray . . ." ['557 Col., 6, lns 35-38]. In electrofusion it is the charge that causes the two cells to fuse while in the '557 it is the high speed impact between a cell and the particles of the spray.

Accordingly, Applicants respectfully request withdrawal of the rejection and allowance of the claims.

CONCLUSION

In view of the above amendment and remarks, Applicants believe the pending application is in condition for allowance.

Should the Examiner wish to discuss any of the amendments and/or remarks made herein, the undersigned attorney would appreciate the opportunity to do so.

The Director is hereby authorized to charge any credits or deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 04-1105, under Order No. 59760 (47137).

Respectfully submitted,

Date: November 5, 2007

/Jeffrey L. Kopacz/
Jeffrey Kopacz
Registration No.: 54,744
EDWARDS ANGELL PALMER & DODGE, LLP
P.O. Box 55874
Boston, MA 02205
Telephone No.: (617) 239-0719
Attorneys/Agents For Applicant

Customer No.: 21874